Agilent HPLC-Chip: G4240-62001 ZORBAX 300SB-C18

Data sheet for the HPLC-Chip separation and enrichment column

General Description:

The G4240-62001 HPLC-Chip is the standard chip for one-dimensional separations. It is comprised of a 40 nL enrichment column and a 75 µm x 43mm separation column which is packed with Zorbax 300SB-C18 5 µm material. The HPLC-Chip is made from biocompatible polyimide and the functionality of this chip is equivalent to conventional nanospray LC/MS. ZORBAX 300SB-C18 is a micro particulate column packing designed specifically for reversed-phase HPLC of peptides and proteins. The StableBond packing is made by chemically bonding a sterically protected octadecyl stationary phase to a specially prepared, ultra-high-purity, ZORBAX, porous-silica microsphere. The special ZORBAX silica support is designed to reduce or eliminate strong adsorption of basic compounds. The densely covered, sterically protected diisobutyl n-octadecyl stationary phase is chemically stable and gives longer column life. As a result, ZORBAX 300SB-C18 is a stable, reversed-phase packing at low pH that can be used for peptide maps, and separation of synthetic and natural peptides and proteins. It is particularly well suited for use with aggressive mobile phases (e.g., pH < 2, high ionic strength, ion-pair additives, TFA, etc.) since the steric protection of the bonded phase resists degradation caused by such mobile phases. This characteristic is particularly important for use in methods that need long-term stability and reproducibility. ZORBAX 300SB-C18 is well suited to applications that utilize high-sensitivity detectors that require low backgrounds (e.g., mass spectrometers). The uniform, spherical, ZORBAX 300SB-C18 particles have a controlled pore size of 300Å. The HPLC-Chip enrichment and analytical columns are packed using a proprietary, high-pressure, slurry-packing technique to give optimum column stability.

Safety Considerations

All points of connection in liquid chromatographic systems are potential sources of leaks. Users of liquid chromatographic equipment should be aware of the toxicity or flammability of their mobile phases.

Operational Guidelines

Inspect the HPLC-Chip immediately upon its arrival. If there are any signs of damage, notify your local Agilent representative at once. Scratches and other markings may be visible in the rotor area of the HPLC-Chip. Do not attempt to remove them. This is normal and occurs during the manufacturing final test. Record the HPLC-Chip type and serial number, purchase date and operating limits. Keep a record of chip usage along with your test chromatogram. This record will be invaluable in diagnosing chromatographic problems.

A new column contains acetonitrile. Columns should not be left in low or elevated pH solvent or at elevated temperature when not in use.

ZORBAX 300SB-C18 is compatible with water and all common organic solvents. Care should be taken not to pass any mobile phase through the column that might cause a precipitate.

The appearance of scratches and markings in the rotor area following use of the HPLC-Chip is normal and does not have any impact on chip performance.

Maximum operating pressure for the HPLC-Chip is 150 bar (2175 psi).

Avoid use of mobile phases below pH 0.8 or above pH 8.0. Solvents with pH > 8.5 must not be used as they dissolve the quartz capillaries in the nano LC system and Chip Cube.

Avoid touching the exposed polyimide surfaces of the HPLC-Chip.

Do not manually extend the HPLC-Chip tip outside of the chip holder as this may result in tip damage and poor spray performance.

When not in use, always store the HPLC-Chip in its storage box to avoid dust or other foreign particle contamination.

NOTE: StableBond columns are designed for high stability at low pH (e.g., pH < 5). However, all silica-based packings have some solubility in pH > 6 aqueous mobile phases. Therefore, when using silica-based columns under conditions of pH > 6, maximum column lifetime is obtained by operation at low temperatures (< 40°C) using low buffer concentrations in the range of 0.01 to 0.02M. Column stability at pH > 6 is also enhanced by avoiding phosphate and carbonate buffers [ref.: H.A. Claessens, M.A. van Straten, and J.J. Kirkland, J Chromatogr. (A), 728 (1996) 259].

Special Considerations for use of the HPLC-Chip

The Agilent HPLC-Chip is specifically designed for use with the Agilent G4240A HPLC-Chip/MS Cube interface. Attempting to use the HPLC-Chip with other devices can damage the chip. Use of the HPLC-Chip with the G4240A HPLC-Chip/MS Cube is described in the Chip Cube user manual, P/N G4240-90000. Descriptions for connection and precautions described here refer to this system.

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Special Operational Guidelines for the Agilent HPLC-Chip

Conditioning. Always purge the HPLC-Chip with a strong solvent like 100% acetonitrile or methanol prior to first usage.

Flow rate. The flow rate for optimal column efficiency for the HPLC-Chip separation column is proportionately smaller than that of a standard bore column due to the smaller diameter. Typical flow rates recommended for the HPLC-Chip separation column are 0.2 –0.6 µl/min.

Sample solvent. Since the enrichment and separation columns are used exclusively for gradient elution of peptides or proteins, the solutes should be dissolved in the starting solvent or in a solvent with weaker elution strength than the starting solvent.

Injection Volume. The volume of sample injected will affect the efficiency of the column. Sample-volume requirements though are less critical if the initial capacity factor of the solutes in the sample solvent is higher than in the starting solvent. In these cases, it is possible to inject several column volumes of sample (up to 1-2 µL), concentrating it on the enrichment column. The sample is then forward/backward flushed to the separation column and eluted at appropriate gradient compositions with low peak volumes.

Column Pressure. The very narrow I.D. capillaries used in the system for connections to the nanoLC pump and autosampler may occasionally plug despite the very smooth inner surface of such capillaries. It is good practice and strongly recommended to monitor and log the HPLC-Chip pressure regularly under standard conditions. In case there is a deviation of more than 10% from previously logged values, one of the transfer capillaries may be plugged. In such case, proceed as described in the column care section.

HPLC-Chip Column Care

The HPLC-Chip enrichment column has a screen with a nominal porosity of 3 µm. Samples that contain particulate matter larger than 3 µm may plug the enrichment column inlet screen and should be filtered before injection into the column. With samples of high viscosity or samples that tend to precipitate the use of an in-line prefilter is highly recommended.

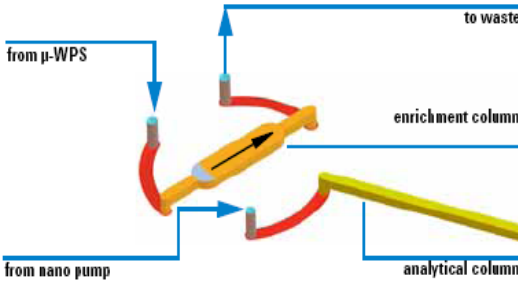
If solvent flow through the enrichment column appears to be restricted (high pressure), check first to see that solvent flow is unobstructed up to the HPLC-Chip inlet (connection capillaries). If the HPLC-Chip has the restriction, there may be particulate matter on the inlet screen. An initial attempt should be made to remove any inlet debris by back-flushing the mobile phase through the enrichment column. If this fails to return the enrichment column to near its original backpressure, the HPLC-Chip requires replacement.

HPLC-Chip Storage

The HPLC-Chip columns (enrichment and analytical) may be safely stored for short periods in most mobile phases. Long term storage of silica-based, bonded phase columns should be in a pure organic solvent, preferably an aprotic liquid such as 100% acetonitrile. If the column has been previously used with a buffered mobile phase, the buffer should first be removed by purging both columns with 20-30 column volumes of a 50/50 mixture of methanol or acetonitrile and water, followed by 20-30 column volumes of the pure solvent. The enrichment column volume is 40 nL, the separation column volume is 160 nL.

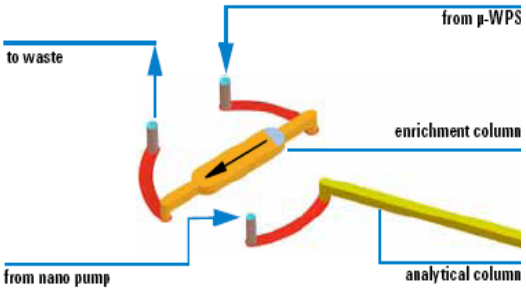
Flow path diagram for G4240-62001 HPLC-Chip

Forward flush mode:



Port 6 of the HPLC-Chip is connected to the µ-well-plate sampler. The loading solvent is directed to waste through port 5. Port 2 is connected to the nano-pump.

Backward flush mode:



Port 5 of the HPLC-Chip is connected to the µ-well-plate sampler. The loading solvent is directed to waste through port 6. Port 2 is connected to the nano-pump

HPLC-Chip radio frequency identification tag

The HPLC-Chip contains a radio frequency (RF) identification (ID) tag in the chip handle. The RF ID tag contains information on the HPLC-Chip layout. The information on the RF ID tag is automatically downloaded and updated by the system software from the HPLC-Chip Cube interface. Information stored on the chip RF ID tag:

- Product number
- Serial number#
- Revision
- Max. Pressure Limit
- Number of injections
- Time stamp of first usage
- Time stamp of last usage
- Total time in operation
- User comment field

G4240-90201

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